PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A01H 1/00, 5/00, 5/10, C12N 5/00, 15/00

A1

(11) International Publication Number: WO 94/22289

(43) International Publication Date: 13 October 1994 (13.10.94)

(21) International Application Number: PCT/US94/03522

(22) International Filing Date: 30 March 1994 (30.03.94)

(30) Priority Data:
105,243 31 March 1993 (31.03.93) IL

(71) Applicant *(for all designated States except US)*: PERI DEVELOPMENT APPLICATIONS (1985) LTD. [IL/IL]; The Volcani Center, P.O. Box 6, 50250 Bet Dagan (IL).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: SCHAFFER, Arthur [US/IL]; 16 Hazait Street, Mobile Post Modiin, 71907 Hashmonaim (IL).

(74) Agents: GALLOWAY, Peter, D. et al.; Ladas & Parry, 26 West 61st Street, New York, NY 10023 (US).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: METHOD FOR BREEDING TOMATOES WITH SUPERIOR TASTE CHARACTERISTICS AND PRODUCT OF THE METHOD

(57) Abstract

A method for breeding tomato plants that produce tomatoes having superior taste characteristics including the steps of, crossing at least one *Lycopersicon esculentum* plant with a *Lycopersicon* spp. to produce hybrid seeds, collecting the hybrid F_1 seeds, growing plants from the F_1 seeds, pollinating the F_1 plants, collecting the hybrid seeds produced by the F_1 plants, growing plants from the seeds produced by the F_1 plants, measuring sucrose, glucose and fructose content of ripe fruit produced from the plants grown from the seeds of the F_1 plants; and selecting plants with tomato fruits having desired characteristics including a fructose/glucose ratio greater than 1.8 and fructose levels higher than 1.3 % on a fresh weight basis.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

METHOD FOR BREEDING TOMATOES WITH SUPERIOR TASTE CHARACTERISTICS AND PRODUCT OF THE METHOD

The present invention relates to a method of breeding tomatoes having superior taste characteristics and to tomatoes having superior taste characteristics and to products of the method.

Taste characteristics are a major determinant of fruit quality for both processing and fresh market tomatoes (Stevens, M.A., 1986, Inheritance of Tomato Fruit Quality Components, Plant Breeding Reviews, 4, 274-310). One of the major components of taste in tomatoes is soluble sugar content.

The soluble sugar content of all known commercial cultivars of tomato (Lycopersicon esculentum Mill.) primarily includes the hexose sugars glucose and fructose in ratios of approximately 1:1 to 1:1.5 (Davies, J.N and Hobson, G.E., 1981, The Constituents of Tomato Fruit - The Influence of the Environment, Nutrition and Genotype, CRC Critical Reviews in Food Science and Nutrition, 15: 205 - 280; Davies, J.N. and Kempton, R.J., 1975, Changes in the Individual Sugars of Tomato Fruit During Ripening, J.Sci. Fd. Agric., 26: 1103 - 1110).

In commercial L sculentum cultivars

the disaccharide sucrose is also present, but at concentrations rarely exceeding 0.5% on a fresh weight basis. Certain wild species of Lycopersicon such as L. hirsutum and L. chmielewskii, accumulate high concentrations of sucrose, which may reach 4% on a fresh weight basis (Miron, D. and Schaffer A.A., 1991, Sucrose Phosphate Synthase, Sucrose Synthase and Invertase Activities in Developing Fruit of Lycopersicon esculentum and the Sucrose Accumulating Lycopersicon hirsutum, Plant Physiol. 95: 623 - 627 and Yelle S. et al., 1988, Sink Metabolism in Tomato Fruit. III. Analysis of Carbohydrate Assimilation in Wild Species, Plant Physiol. 87: 737 - 740). Some of these species, in addition, have a fructose to glucose ratio of more than 1.5; however, fructose and glucose levels in the fruit of these species is very low, below 1.3% each on a fresh weight basis (Davies, J.N. and Kempton, R.J., 1975, Changes in the Individual Sugars of Tomato Fruit During Ripening, J. Sci. Fd. Agric., 26: 1103 - 1110; Davies, J.N., 1966, Occurrence of Sucrose in the Fruit of Some Species of Lycopersicon, Nature, 209, 640 - 641).

Typically, plant breeders seek to increase the sweetness component of tomato flavor by increasing total soluble solids (TSS). TSS is typically estimated by a refractometic determination of a sample of juice and is expressed in °BRIX. The measurement of °BRIX, however, does not differentiate between the component sugars. Selections have recently been made for sucrose accumulating tomatoes (Yelle, S., 1991, Sink Metabolism in Tomato Fruit IV Genetic and Biochemical Analysis of Sucrose

Accumulation, Plant Physiol. 95, 1026 - 1035). Fructose, however, is twice as sweet per unit weight as glucose and 50% sweeter than sucrose (Biester, A.M. et al., 1925, Carbohydrate Studies. I. The Relative Sweetness of Pure Sugars, Amer. J. Physiol. 73: 387 - 400) giving a tomato with a high relative fructose content distinct advantages in terms of superior taste characteristics.

The present invention seeks to provide an improved method for breeding tomato plants having superior taste characteristics and products of the method.

There is thus provided in accordance with the present invention a method for breeding tomato plants that produce tomatoes having superior taste characteristics including the steps of, crossing at least one Lycopersicon esculentum plant with a Lycopersicon spp. to produce hybrid seeds, collecting the hybrid (F_1) seeds, growing plants from the F_1 seeds, pollinating the F_1 plants, collecting the hybrid seeds produced by the F_1 plants, growing plants from the seeds produced by the F_1 plants, measuring sucrose, glucose and fructose content of ripe fruit produced from the plants grown from the seeds of the F_1 plants; and selecting plants with tomato fruits having desired characteristics including a fructose/glucose ratio greater than 1.8 and fructose levels higher than 1.3% on a fresh weight basis.

In accordance with a preferred embodiment of the present invention the method for breeding tomato plants additionally includes the steps of crossing plants which have been selected according to the method of claim 1 with a Lycopersicon plant and selecting plants with

tomato fruits having desired characteristics including a fructose/glucose ratio greater than 1.8 and fructose levels higher than 1.3% on a fresh weight basis.

In accordance with a further preferred embodiment of the present invention the steps of crossing and selecting are repeated at least once.

In accordance with yet a further preferred embodiment of the present invention the method for breeding tomato plants additionally includes the steps of selfing, at least once, the plants, and selecting plants with tomato fruits having desired characteristics including a fructose/glucose ratio greater than 1.8 and fructose levels higher than 1.3% on a fresh weight basis.

In accordance with still a further preferred embodiment of the present invention the Lycopersicon spp. plant having a fructose: glucose ratio greater than 1.8 is a Lycopersicon hirsutum plant.

In accordance with still another preferred embodiment of the present invention crossing includes sexual crossing.

In accordance with yet another preferred embodiment of the invention crossing includes asexual crossing.

In accordance with a further preferred embodiment of the invention asexual crossing includes somatic cell hybridization.

In accordance with a still further preferred embodiment of the invention the step of pollinating includes self pollination.

In accordance with yet a further preferred embodiment of the invention the step of pollination includes back crossing with a

Lycopersicon esculentum plant.

In accordance with still another preferred embodiment of the present invention the method for breeding tomato plants additionally includes the step of propagating the plants with tomato fruits having the desired characteristics.

In accordance with yet another preferred embodiment of the present invention the step of propagating includes the step of vegetative propagation.

In accordance with a further preferred embodiment of the present invention the step of propagating includes the step of propagation by seed.

In accordance with a still further preferred embodiment of the present invention there is provided a tomato plant produced according to the method described hereinabove.

In accordance with yet a further preferred embodiment of the present invention there is provided a tomato fruit produced by a tomato plant produced according to the method described hereinabove.

In accordance with another preferred embodiment of the present invention there is provided tomato seeds which when grown yield a tomato plant produced according to the method described hereinabove.

There is also provided in accordance with the present invention a heterozygous tomato plant producing fruit having a fructose/glucose ratio greater than 1.8 and fructose levels higher than 1.3% on a fresh weight basis.

In accordance with a preferred embodiment of the present invention the tomato plant includes t mato fruit.

In accordance with a further preferred embodiment of the present invention the tomato plant includes tomato seeds which when grown yield the tomato plant described hereinabove.

Reference is now made to a method for breeding tomato plants that produce tomatoes having superior taste characteristics including fructose levels, i.e. greater than 1.3% on a fresh weight basis and a fructose/glucose ratio higher than 1.8. The elevated fructose/glucose ratio providing a sweeter taste than that found in tomatoes having a similar amount of total sugars but with a normal fructose to glucose ratio.

The method for breeding tomato plants includes first hybridizing at least one Lycopersicon esculentum plant with a Lycopersicon hirsutum plant. The fruits of the L esculentum plants are then allowed to ripen and the hybrid (F_1) seeds are collected.

The collected F_1 seeds are then planted. F_1 plants are grown and then allowed to self pollinate. The self pollinated flowers are then allowed to produce ripe fruits and the F_2 seeds are collected. These seeds are then planted. Plants grown from these seeds are allowed to produce fruits which are harvested.

The harvested fruits are then analyzed for °BRIX, sucrose, glucose and fructose content, using methods described below, and plants with tomato fruits having desired characteristics including a fructose/glucose ratio greater than 1.8 and fructose levels higher than 1.3% on a fresh weight basis are selected.

The selected plants may then be propagated for use by vegetative propagation

fruit was harvested and individually analyzed for TSS (°BRIX), and soluble sugar content, as described hereinbelow.

Only 25 of the interspecific F_2 plants freely produced fruit. Seed was collected from all plants. The most promising four plants which showed desired characteristics including a fructose/glucose ratio greater than 1.8 and fructose levels higher than 1.3% on a fresh weight basis were used to produce F_3 populations. Again F_3 seed was sown and the plants were allowed to self pollinate and produce fruit and ripe fruit of individual plants was analyzed as described hereinbelow.

MEASUREMENT OF °BRIX AND SOLUBLE SUGAR (SUCROSE, GLUCOSE AND FRUCTOSE) OF MATURE FRUIT PERICARP

Individual fruits were harvested. The juice was manually expressed from a portion of the fruit pericarp and a few drops placed on a refractometer and °BRIX values read. An additional portion of the fruit tissue was placed in 80% ethyl alcohol and heated to 70°C in order to stop enzymatic activity and extract the soluble sugars. Soluble sugars were extracted three times in successive changes of 80% alcohol which was then evaporated.

The sugars were then dissolved in double distilled water, centrifuged at 5,000 rpm in an eppendorf centrifuge tube for 15 min. to remove debris and a 0.5 ml aliquot passed through a 0.45 micron filter in preparation of High Pressure Liquid Chromotography (HPLC) analysis. HPLC analysis was performed using a BioRad (Richmond, CA, USA) Fast Carbohydrate column for the separation of glucose, fructose and sucros according to the manufacturers

methods such as micropropagation or by sexual propagation methods. The selected plants may also be selfed for at least one generation or crossed with other L esculentum cultivars to create varieties which incorporate characteristics other than the high fructose/glucose ratios selected for.

These derived plants may then be propagated either vegetatively or by seed based propagation.

Reference is now be made to the following example which illustrates the invention.

EXAMPLE 1

HYBRIDIZATION

Ten plants of the L esculentum breeding line 1630 (a Volcani Institute male sterile breeding line) were crossed with the wild species L. hirsutum (LA 1777) using the L. esculentum breeding line as the female parent. The L. esculentum parent contained approximately equimolar concentrations of glucose and fructose (see Table 1). The L. hirsutum parent (LA 1777) accumulated sucrose but had a low concentration of fructose/glucose and a fructose glucose ratio of 2.3 but with less than 1.3% fructose on a fresh weight basis (see Table 1).

The fruits of the L esculentum plants were then allowed to ripen and the hybrid (F_1) seeds collected. Hybrid F_1 seed was sown and plants grown. The plants were allowed to self pollinate and after the ripening of the fruit F_2 seed was collected.

The F_2 seeds were sown and about 350 F_2 plants were grown and allowed to self pollinate. The fruits were allowed to ripen and fruit from each individual plant which produced

instructions. The sugars were identified and quantified according to the chromatographic behavior of standards for the sugars which were obtained from Sigma (St. Louis, MO, USA).

The sucrose, glucose and fructose values of fruit of selected F_3 plants are shown in Table 1.

Table 1

Percent Gram Fresh Weight of Individual Sugars in Selected Ripe F_3 Tomato Fruit

Plant No.	Suc.	Glu.	Fru.	Total Sugars	Fru/Glu ratio
Parents					
L. sculentum 1630	.20	1.10	1.40	2.70	1.27
L. hirsutum LA 1777	4.90	.30	.70	5.90	2.30
F_3 Plants					
200-01	.97	.66	3.11	4.74	4.71
201-10	2.50	1.45	2.87	6.82	1.98
203-07	.58	.85	3.58	5.01	4.21
203-10	.28	1.30	3.37	4.95	2.59

It will be appreciated by persons skilled in the art that the present invention is not limited to what has been particularly shown and described hereinabove. Rather the scope of the present invention is defined only by the claims which follow:

C L A I M S

1. A method for breeding tomato plants that produce tomatoes having superior taste characteristics comprising the steps of:

crossing at least one Lycopersicon esculentum plant with a Lycopersicon spp. to produce hybrid seeds;

collecting the hybrid (F_1) seeds;

growing plants from the F_1 seeds;

pollinating the F_1 plants;

collecting the hybrid seeds produced by the F_1 plants;

growing plants from the seeds produced by the F_1 plants;

measuring sucrose, glucose and fructose content of ripe fruit produced from the plants grown from the seeds of the F_1 plants; and

selecting plants with tomato fruits having desired characteristics including a fructose/glucose ratio greater than 1.8 and fructose levels higher than 1.3% on a fresh weight basis.

2. A method according to claim 1, and additionally comprising the steps of:

crossing plants which have been selected according to the method of claim 1, with a Lycopersicon plant and

selecting plants with tomato fruits having desired characteristics including a fructose/glucose ratio greater than 1.8 and fructose levels higher than 1.3% on a fresh weight basis.

3. A method according to claim 2, wherein the steps of crossing and selecting are repeated at least once.

4. A method according to any of the above

claims, wherein crossing includes sexual crossing.

5. A method according to any of claims 1 - 3, wherein crossing includes asexual crossing.

6. A method according to claim 5, wherein asexual crossing includes somatic cell hybridization.

7. A method according to claim 1, wherein the step of pollinating includes self pollination.

8. A method according to claim 1, wherein the step of pollination includes back crossing with a Lycopersicon esculentum plant.

9. A method according to any of the above claims, wherein the Lycopersicon spp. plant having a fructose : glucose ratio greater than 1.8 is a Lycopersicon hirsutum plant.

10. A method according to any of the above claims, and additionally comprising the step of propagating the plants with tomato fruits having the desired characteristics.

11. A method according to claim 10, wherein the step of propagating includes the step of vegetative propagation.

12. A method according to claim 10, wherein the step of propagating includes the step of propagation by seed.

13. A tomato plant produced according to the method of any of claims 1 - 12.

14. A tomato fruit produced by a tomato plant in accordance with claim 13.

15. Tomato seeds which when grown yield a tomato plant in accordance with claim 13.

16. A heterozygous tomato plant producing fruit having a fructose/glucose ratio greater than 1.8 and fructose levels high r than 1.3% on

a fresh weight basis.

17. Tomato seeds which when grown yield a tomato plant in accordance with claim 16.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03522

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A01H 1/00, 5/00, 5/10; C12N 5/00, 15/00
US CL :47/58; 435/172.2, 240.45; 800/200, 255

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 47/58; 435/172.2, 240.45; 800/200, 255

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,940,839 (BRAVO ET AL) 10 July 1990, column 2, lines 12-25 and 44-61.	5, 6, 11
Y	HORTSCIENCE, Volume 23, No. 3, issued June 1988, A. C. Meyer et al, "Estimating Heritability and the Number of Genes Involved in Controlling Fructose and Glucose Concentrations in Tomato (Lycopersicon Esculentum)", page 767, abstract no. 346, see entire abstract.	1-17
Y	NATURE, Volume 209, issued 05 February 1966, J. N. Davies, "Occurence of Sucrose in the Fruit of Some Species of *Lycopersicon*", pages 640-641, see page 641.	1-17

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 June 1994	Date of mailing of the international search report JUN 27 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID T. FOX Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PLANT PHYSIOLOGY, Volume 95, No. 2, issued February 1991, D. Miron et al, "Sucrose Phosphate Synthase, Sucrose Synthase, and Invertase Activities in Developing Fruit of *Lycopersicon esculentum* Mill. and the Sucrose Accumulating *Lycopersicon hirsutum* Humb. and Bonpl.", pages 623-627, see page 625.	1-17
Y	PLANT PHYSIOLOGY, Volume 95, No. 4, issued April 1991, S. Yelle et al, "Sink Metabolism in Tomato Fruit. IV. Genetic and Biochemical Analysis of Sucrose Accumulation", pages 1026-1035, see page 1027.	1-17